

MODIFICATION OF SEA ANEMONE BEHAVIOR BY SYMBIOTIC ZOOXANTHELLAE: PHOTOTAXIS

VICKI BUCHSBAUM PEARSE¹

Hopkins Marine Station, Pacific Grove, California 93950

Symbiosis between an animal host and a photosynthetic symbiont is unlikely unless the animal spends considerable time in the sun. So it is not surprising that a variety of invertebrates with unicellular endosymbiotic algae display positive phototaxis. Probably the first report of phototaxis in an algae-bearing invertebrate is Trembley's (1744) observation that green hydras gather on the lighted side of a glass vessel. Later investigations show, however, that other, non-symbiotic hydra species (Wilson, 1891) and green hydras which have lost their symbionts (Whitney, 1907) also respond positively to light. The acel flatworms *Convoluta roscoffensis* and *C. convoluta* show phototactic behavior correlated with their usual habitats (Keeble, 1910). Again, the symbiotic algae are not essential to the response. The action spectrum for phototaxis does not correspond to that of chlorophyll, and light apparently is received by the carotenoid-containing eyespots of the animal. Even worms just a few hours old and still white respond phototactically. Vandermeulen, Davis, and Muscatine (1972) mention that *Placobranchus ianthobapsus*, a saccoglossan opisthobranch gastropod with functional chloroplasts derived from siphonaceous green algae, displays positive phototaxis even when chloroplast photosynthesis is effectively eliminated by chemical inhibition. In other cases, however, phototaxis appears to depend on the intact, functional symbiosis. The ciliate *Paramecium bursaria* with zoochlorellae shows positive phototaxis, and the action spectrum of the response corresponds to the absorption spectrum of chlorophyll (Engelmann, 1882). *P. bursaria* without algal symbionts does not show positive phototaxis (Siegel, 1960), nor do any of the other, non-symbiotic species of *Paramecium* show directed light responses (Gelber, 1956; Halldal, 1964).

Besides the green hydras, there are several other examples of tactic responses to light among algae-bearing cnidarians. Coral planulas containing zooxanthellae swim toward light; those without zooxanthellae display no light response (Kawaguti, 1941, 1944; Atoda, 1953). Zahl and McLaughlin (1959) report that *Condylactis* sp., a Caribbean sea anemone with zooxanthellae, moves out of direct sunlight into shade, but they did not establish whether the anemones respond positively to light of lower intensity. Specimens of *Condylactis* that are kept in the dark lose their symbionts and also lose their phototactic response. Cotte (1921, 1922) describes inconsistent phototactic behavior in *Anemonia sulcata*, a European sea anemone with zooxanthellae. Of the few other sea anemones in which phototaxis has been tested, all non-symbiotic species, most appear either indifferent or negative to light (*e.g.*, Hargitt, 1907; Parker, 1917; Ottaway, 1973).

¹ Present address: Division of Natural Sciences, University of California, Santa Cruz, California 95064.

Most littoral anemones live in shaded habitats. However, the two algae-bearing sea anemones of the west coast of North America, *Anthopleura elegantissima* (Brandt, 1835) and *A. xanthogrammica* (Brandt, 1835), are unusual in distribution; most occupy rocks and tidepools in full sun. This unusual habit, apparently associated with the symbiotic relationship, led me to suspect that these anemones might show responses to light which were different from those of most actinians.

Anthopleura elegantissima is particularly suitable for studying possible relationships between behavior and symbiosis because the animals are hardy and abundant; exposed for collection at almost any low tide; mostly conveniently small (although solitary individuals may be as large as many large *A. xanthogrammica*); and reproduce asexually by dividing, forming distinct clonal beds with tens to hundreds of genetically identical individuals (see Francis, 1973). Clones can be found in light conditions grading from direct sunlight to deep shade, with the number of algal symbionts correspondingly graded. It is possible to introduce zooxanthellae into anemones that lack them (Trench, 1971c); and individuals with algae can be made to lose their symbionts, yet remain apparently active and healthy (see Methods). As part of a more extensive study on the symbiosis of *A. elegantissima* with zooxanthellae (Buchsbaum, 1968; Pearse, 1974), I have tested anemones of this species, with and without symbiotic algae, for evidence of phototactic behavior and have tried to discover whether their behavior and symbiosis might be related.

METHODS

Specimens of the sea anemone *Anthopleura elegantissima* with abundant symbiotic algae were collected from two rocky intertidal areas in central California. Specimens with few and no zooxanthellae were collected from intertidal rocks or cement pillars beneath the cannery buildings, Cannery Row, Monterey. Only golden-brown dinoflagellate zooxanthellae were present as algal symbionts in the anemones.

Tests for phototaxis

The sea anemones were tested for phototaxis in shallow seawater tables. Eight 112-cm Sylvania Lifeline fluorescent tubes were suspended 30 cm above the table; they were left on continuously during tests. Incident illumination at the center of the table was 700 foot-candles, as measured with a Photovolt 200 photometer, also used for intertidal light measurements. A continuous flow of sea water along the table maintained the temperature at $14^{\circ}\text{C} \pm 1^{\circ}\text{C}$. The water was approximately 4 cm deep and covered the anemones. A wooden board supported just above the surface of the water shadowed half of the experimental area. The total area was 22 cm across by 125 cm long.

At the beginning of each of the 19 experiments, 25–70 anemones were placed in the table under uniform illumination. After the anemones had all attached to the surface of the table, their positions were recorded, and the wooden board was arranged. The positions of the anemones, along the table as well as with

respect to the lighted and shadowed halves, were then recorded at intervals, usually daily. The day after the wooden board was set in place was counted as the first day. The small number of animals occasionally found on the borderline between lighted and shadowed areas was divided equally between the two scores. The anemones were not fed.

Elimination of zooxanthellae

Maintenance in the dark, used successfully to eliminate symbiotic algae from various invertebrate hosts (see, *e.g.*, Zahl and McLaughlin, 1959), was not very satisfactory for *A. elegantissima* because of the long time required. The anemones were maintained in a darkroom in running sea water and exposed to dim light for only a few minutes every few weeks, in order to take sample bits of tissue, cut from the tentacles and sides of the column. Only after 12 weeks in the dark did most of the tissue samples clearly have fewer zooxanthellae than at the beginning; after nearly three times this long (34 weeks), a few zooxanthellae were still found. When the treatment was terminated at 48 weeks, no zooxanthellae were found in the tissue samples, nor did zooxanthellae return when these animals were subsequently kept in the light.

The most satisfactory method found for ridding the anemones of their symbiotic zooxanthellae was exposure to elevated temperatures. The anemones were kept in warm sea water for one to several days, then put back into running sea water at 13°–15° C for 2–3 weeks. During this time they egested mucus-wrapped pellets of zooxanthellae until no algae were found in their tissues. The anemones appeared healthy and normal throughout the treatment, as long as the heated sea water was aerated continuously and changed frequently. The effectiveness of exposure to temperatures from 25°–37° C, for varying periods of time, was tested (see Buchsbaum, 1968 for details). The time and temperature ranges that were both effective and well-tolerated by the anemones were very narrow; 30°–32° C for 48 hours was most successful. Such heat-treated anemones have been kept in the light for more than a year, never regaining any zooxanthellae, and this appears to be a convenient and reliable method of obtaining anemones without zooxanthellae for experimental purposes.

RESULTS

Sea anemones with zooxanthellae

Sea anemones with zooxanthellae, gathered from sunny intertidal habitats, always distributed themselves in the lighted half of the experimental area, whether the animals were initially equally distributed between the lighted and shadowed halves (Figure 1A) or placed entirely in the shadowed area (Figure 2A). The time course of the response varied in different experiments. The animals represented in Figure 1A were the slowest observed; 95% were found in the lighted area after 13 days. In another, similar experiment 93% were found in the lighted area after only 4 days. In the experiment represented in Figure 2A, although all

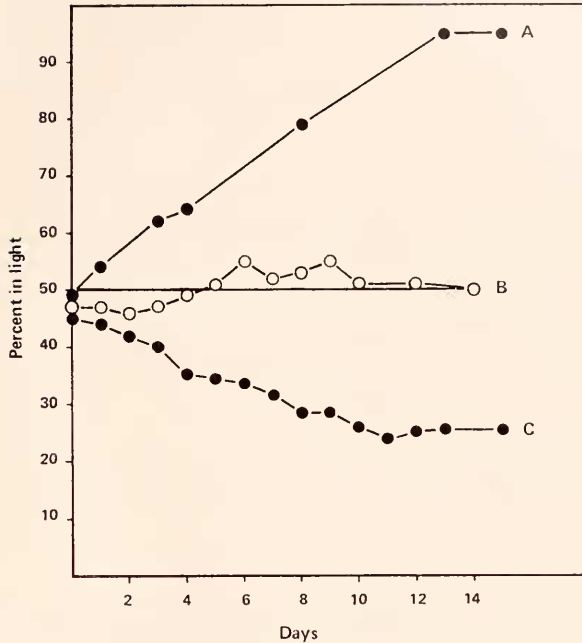


FIGURE 1. Percentage of anemones in lighted half of seawater table, after being initially randomly distributed. At 2 weeks: (A.) Anemones with zooxanthellae, from a sunny habitat, showed a significant positive light response ($\chi^2 = 30.4$, $P < 0.001$, $N = 38$). (B.) Anemones without zooxanthellae showed no significant light response ($\chi^2 = 0$, $P > 0.99$, $N = 51$); zooxanthellae were eliminated from these animals after exposure to elevated temperature. (C.) Anemones with zooxanthellae, from a shaded habitat (about 9% of light intensity measured in open intertidal), showed a significant negative light response ($\chi^2 = 16.0$, $P < 0.001$, $N = 68$).

of the animals were initially placed in the shadowed half, 92% had moved to the lighted area after only 5 days.

Sea anemones without zooxanthellae

Sea anemones without zooxanthellae distributed themselves randomly with respect to the lighted and shadowed areas. When equal numbers were initially placed in each half of the experimental area, their distribution remained random with respect to light (Figure 1B). When all were initially placed in the shadowed half, they slowly emerged into the lighted area until equally distributed between the lighted and shadowed portions (Figure 2B, C). Again, the time course varied in different experiments.

Sea anemones with few zooxanthellae

Sea anemones gathered from partially shaded intertidal habitats, with incident light down to about 10% of that measured in open intertidal areas, were found to harbor limited numbers of zooxanthellae. Such animals, when tested for photo-

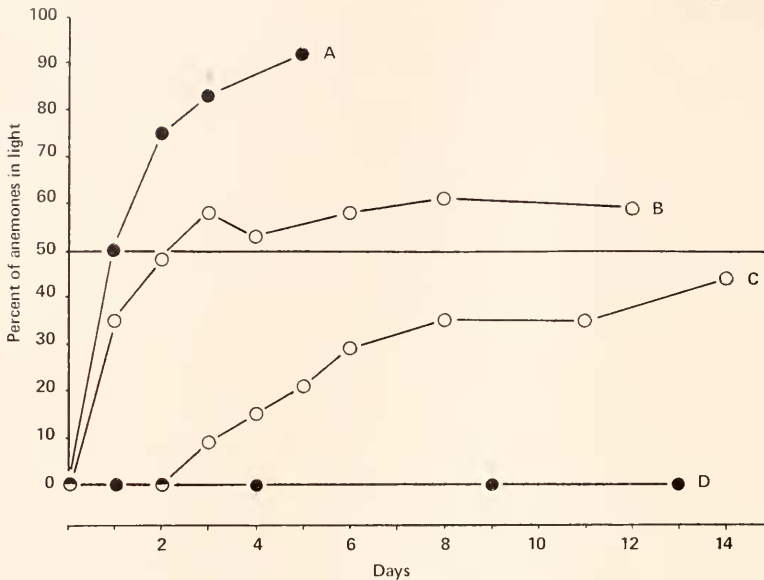


FIGURE 2. Percentage of anemones in lighted half of seawater table, after being initially distributed all in the shadowed half; (A-C) anemones with zooxanthellae when collected, from a sunny habitat, all from the same clone. (A.) Anemones with zooxanthellae showed a significant positive light response (at 5 days: $\chi^2 = 37.2$, $P < 0.001$, $N = 52$). (B.) Anemones having lost zooxanthellae after exposure to elevated temperature showed no significant light response (at 12 days: $\chi^2 = 1.2$, $P = 0.27$, $N = 41$). (C.) Anemones having lost zooxanthellae after maintenance in darkness showed no significant light response (at 14 days: $\chi^2 = 1.1$, $P = 0.30$, $N = 34$). (D.) Anemones with zooxanthellae, from a shaded habitat (about 9% of light intensity measured in open intertidal), showed a significant negative light response (at 13 days: $\chi^2 = 25.0$, $P < 0.001$, $N = 25$).

taxis as above, always occupied the shadowed half of the experimental area. If they were initially equally distributed between lighted and shadowed halves, they moved into the shadowed portion (Figure 1C). If initially placed entirely in the shadowed area, they remained there, none emerging into the light (Figure 2D).

Light intensity

The plasticity of behavior indicated by these results suggested the possibility that the anemones with zooxanthellae which I collected from shaded habitats, and which reacted negatively to the light intensity regularly used in the experiments (Figures 1C, 2D), might respond positively to a lower light intensity. To test this possibility, I selected anemones from a shaded habitat where midafternoon light readings were 200–250 foot-candles. Distributed randomly in a seawater table, these animals showed a negative response to the usual light intensity of 700 foot-candles, moving into the shadowed half of the table (Figure 3, left). After their distribution had remained stable for 2 weeks, the light intensity was reduced

to 250 foot-candles; and within a few days the anemones began to move out into the lighted area, reversing from a negative to a positive response (Figure 3, right).

Elimination of zooxanthellae

All of the results just described suggested that phototaxis in these anemones depends directly on the presence of zooxanthellae. However, since naturally occurring anemones with and without zooxanthellae were collected from different habitats and belonged to different clones, several other hypotheses seemed possible. The response might depend on previous exposure to appropriate light intensities; pigments produced by the anemones only in the light might be involved (see Buchsbaum, 1968). The absence of phototaxis in animals from dark habitats might then reflect only their lack of previous exposure to light and not their lack of zooxanthellae. Alternatively, phototactic behavior might be developed only after the acquisition of zooxanthellae and then retained. In this case, animals without algae might display phototaxis if they had previously harbored zooxanthellae. Finally, phototaxis might have a genetic basis, since an individual that actively orients to light, either as a settling planula or attached adult anemone, is

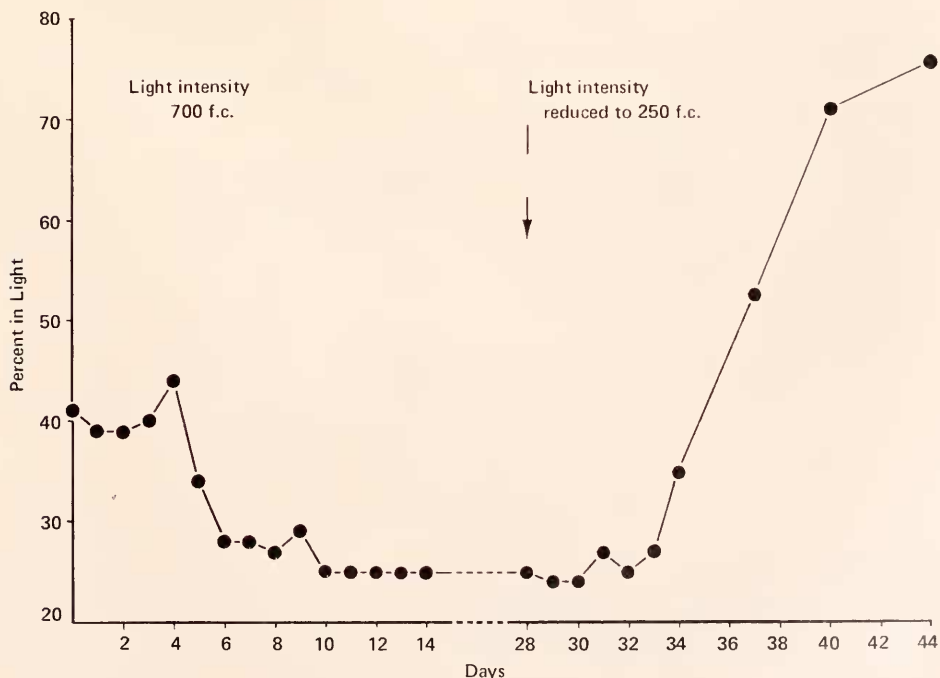


FIGURE 3. Percentage of anemones in lighted half of seawater table, after being initially randomly distributed. Anemones with zooxanthellae, from a shaded habitat (about 47% of light measured in open intertidal), showed a significant negative light response at 700 foot-candles (at 14 days: $\chi^2 = 12.2$, $P < 0.001$, $N = 51$) and later a significant positive light response at 250 foot-candles (after 16 days: $\chi^2 = 14.2$, $P < 0.001$, $N = 51$).

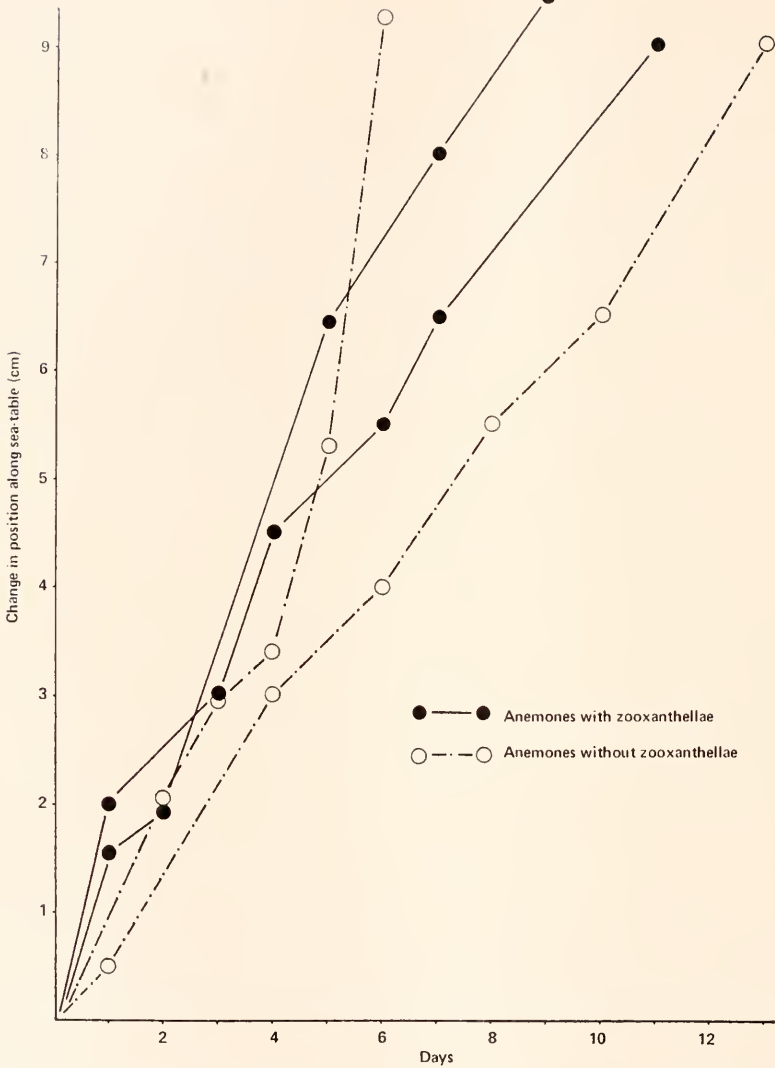


FIGURE 4. Movement of anemones up-current in the seawater tables. Data from 4 experiments with 4 different clones of animals ($N = 34, 30, 38, 32$). Changes in average position along the table (sum of anemone positions/no. of anemones) were taken as an index of movement, since individual anemones could not be conveniently followed.

more likely to become host to light-requiring symbionts than an animal that is indifferent to light.

To test these possibilities, I collected sea anemones from a single asexually produced clone, all with abundant zooxanthellae. Some were kept as controls in

natural light in running sea water at ambient temperature. Others were induced to lose their zooxanthellae by subjecting them briefly to heat or darkness (see Methods). After these treatments, tissue samples examined by light microscopy revealed no zooxanthellae; nor did these anemones ever re-establish symbiosis with algae, although subsequently kept in the light.

All of the anemones—presumably genetically identical, collected from the same small intertidal area, and originally harboring many zooxanthellae—were tested for their response to light. The control individuals with zooxanthellae showed a positive phototactic response (Figure 2A), while those which had lost their zooxanthellae distributed themselves equally between lighted and shadowed areas (Figures 1B, 2B, C). I found no differences in phototactic behavior between anemones occurring naturally without zooxanthellae, collected from dark habitats, and anemones in which I induced loss of zooxanthellae by exposure to heat or darkness.

Activity controls

Another hypothesis considered was that the apparent failure of anemones without zooxanthellae to respond to light might simply reflect inactivity or slowness of movement. However, in experiments in which all animals were initially placed in the shadowed area, anemones with zooxanthellae moved into the light at a rate fast enough to reveal any light response within the duration of the experiment (Figure 2B, C) but with a pattern quite distinct from the positive and negative responses of the anemones with zooxanthellae (Figure 2A, D). Moreover, in all experiments, regardless of light-directed movements across the seawater table, the anemones moved along the table, against the current of sea water. This movement was followed by noting the position of each anemone along the table each time its position with respect to light was recorded. Changes in the positions of the anemones along the table in 4 experiments are represented in Figure 4. Although rates of movement varied in different experiments, there appeared to be no difference in the rates of anemones with and without zooxanthellae.

DISCUSSION

Under the conditions of these experiments, sea anemones with zooxanthellae always displayed phototaxis, either positive or negative depending on the light intensity. Anemones without zooxanthellae—even those that had previously harbored zooxanthellae and that were genetically identical clone-mates of phototactic individuals—never displayed phototaxis, appearing completely indifferent to light and shade. These observations indicate that phototactic behavior in the sea anemone *Anthopleura elegantissima* is not a fixed species character, but rather depends directly on the presence of endosymbiotic zooxanthellae.

The mechanism by which the symbionts influence their anemone hosts is unknown, but possibly involves some metabolite consumed or produced in photosynthesis by the algae. Production of oxygen or any organic compound, removal of carbon dioxide or other metabolic by-products, or release of any stimulant or

inhibitor by the algae could all work in the same way. The difference in light intensity on two sides of the animal would result in different degrees of activity by the algae. The quantities of both animal and algal pigments vary with the light intensities under which the organisms live (Buchsbaum, 1968), and light intensities either higher or lower than the accustomed range might be expected to reduce algal photosynthesis. The gradient thus established within the animal would direct its movement. Determination of the action spectrum for phototaxis would provide evidence for or against some such photosynthetic mechanism. In the ciliate *Paramecium bursaria*, which responds phototactically only under conditions of limited oxygen, the evidence suggests a mechanism involving oxygen production by the symbiotic algae (Jennings, 1915; Stanier and Cohen-Bazire, 1957).

In this context it would be particularly interesting to know the phototactic behavior of specimens of *A. elegantissima* from the northwest coast of North America which are symbiotic with a green unicellular alga. Unlike zooxanthellae, which release approximately 50% of their photosynthetically-fixed carbon to the anemone tissues in a variety of organic compounds (Trench, 1971a, 1971b), these green symbionts fix carbon photosynthetically at appreciable rates but release less than 2% (Muscatine, 1971). Yet in symbiosis with both kinds of algae, the anemone tissues presumably receive oxygen and give up carbon dioxide and other inorganic nutrients to the algae. Tests for phototactic behavior in anemones with the green symbionts might therefore provide clues as to which algal activities influence phototaxis.

The symbiosis between *A. elegantissima* and zooxanthellae was the first in which transfer of photosynthetically-fixed carbon from an alga to its host was directly demonstrated (Muscatine and Hand, 1958). Studies since then have characterized the biochemistry of the algal contribution (Trench, 1971a, 1971b) and have shown that the zooxanthellae slow weight loss of the anemone during starvation in the light (Muscatine, 1961a; Buchsbaum, 1968). The physiological mechanism for a potential selective advantage in the symbiosis is thus well established. Phototaxis seems to be a behavioral mechanism ensuring the effectiveness and continued maintenance of the symbiosis.

If anemones with zooxanthellae are placed in the dark, the number of algal cells and total chlorophyll per unit weight of anemone tissue decrease, eventually to undetectable levels (Muscatine, 1961b; Buchsbaum, 1968). Intertidal anemones in a sunny habitat, if suddenly shaded (for example, by growing seaweeds or by debris), may avoid such reduction in symbionts by positive phototaxis. On the other hand, anemones with zooxanthellae adapted to partially shaded habitats, if suddenly exposed to increased light intensity, also suffer a reduction in numbers of algae and total chlorophyll, the tentacles and oral disk becoming especially pale. After a few days in full sun, microscopic examination of tentacle tissue reveals only rare, bleached algal cells (Buchsbaum, 1968). Shade-adapted anemones may avoid such loss of algae by negative phototaxis. How much these anemones actually move under normal conditions in the intertidal is not known, but their phototactic behavior in laboratory experiments suggests that they may effectively control their light environment in this way.

This study was supported by a predoctoral fellowship from the National Institutes of Health, carried out at the Hopkins Marine Station, Pacific Grove, California, and submitted to Stanford University as part of a doctoral dissertation. I am grateful to the faculty, staff, and students of the Marine Station, especially my advisory committee, Drs. J. H. Phillips, D. P. Abbott, and W. L. Lee; to Dr. J. S. Pearse for reading the manuscript; and to the Division of Natural Sciences, University of California, Santa Cruz for library and secretarial assistance.

SUMMARY

The sea anemone *Anthopleura elegantissima*, with and without endosymbiotic zooxanthellae, was tested for evidence of phototactic behavior. Anemones with zooxanthellae always displayed phototaxis, either positive or negative depending on the experimental light intensity and the light intensity of the habitat from which the animals were taken. Anemones without zooxanthellae—even those that had previously harbored zooxanthellae and that were genetically identical clones of phototactic individuals—never displayed phototaxis, appearing completely indifferent to light and shade.

The results indicate that phototaxis in this sea anemone depends directly on the presence of its symbiotic algae. It is suggested that the flexible phototactic behavior of the anemone may play an important role in favorably regulating the amount of light to which the zooxanthellae are exposed.

LITERATURE CITED

- ATODA, K., 1953. The larval and postlarval development of the reef-building corals. *Sci. Rep. Tohoku Univ., Series 4, Biol.*, **20**: 105–121.
- BUCHSBAUM, V. M., 1968. Behavioral and physiological responses to light by the sea anemone *Anthopleura elegantissima* as related to its algal endosymbionts. *Doctoral dissertation, Stanford University*, 123 pp.
- COTTE, J., 1921. Sur le phototropisme des Actinies. *C. R. Soc. Biol. Paris*, **85**: 188–190.
- COTTE, J., 1922. Études sur les comportements et les réactions des Actinies. *Bull. Inst. Océanogr., Monaco*, **410**: 1–44.
- ENGELMANN, T.W., 1882. Ueber Licht- und Farbenperception niederster Organismen. *Pflüg. Arch. Ges. Physiol.*, **29**: 387–400.
- FRANCIS, L., 1973. Clone specific segregation in the sea anemone *Anthopleura elegantissima*. *Biol. Bull.*, **144**: 64–72.
- GELBER, B., 1956. Investigations on the behavior of *Paramecium aurclia*: The effect of the presence and absence of light on the occurrence of a response. *J. Genet. Psychol.*, **88**: 31–36.
- HALLDAL, P., 1964. Phototaxis in Protozoa. Pages 277–296 in S. H. Hutner, Ed., *Biochemistry and Physiology of Protozoa*. Academic Press, New York.
- HARGITT, C. W., 1907. Behavior of sea-anemones. *Biol. Bull.*, **12**: 274–284.
- JENNINGS, H. S., 1915. *Behavior of the Lower Organisms*. Columbia University Press, New York, 366 pp.
- KAWAGUTI, S., 1941. On the physiology of reef corals. V. Tropisms of coral planulae, considered as a factor of distribution of the reefs. *Palao Trop. Biol. Stat. Stud.*, **2**: 319–328.
- KAWAGUTI, S., 1944. Zooxanthellae as a factor of positive phototropism in those animals containing them. *Palao. Trop. Biol. Stat. Stud.*, **2**: 681–682.
- KEEBLE, F., 1910. *Plant-Animals: A Study in Symbiosis*. Cambridge University Press, 163 pp.

- MUSCATINE, L., 1961a. Symbiosis in marine and fresh-water coelenterates. Pages 255-264 in H. M. Lenhoff and W. F. Loomis, Eds., *The Biology of Hydra*. University of Miami Press, Coral Gables, Florida.
- MUSCATINE, L., 1961b. Some aspects of the relationship between a sea anemone and its symbiotic algae. *Doctoral dissertation, University of California, Berkeley*, 100 pp.
- MUSCATINE, L., 1971. Experiments on green algae coexistent with zooxanthellae in sea anemones. *Pac. Sci.*, **25**: 13-21.
- MUSCATINE, L., AND C. HAND, 1958. Direct evidence for transfer of materials from symbiotic algae to the tissues of a coelenterate. *Proc. Nat. Acad. Sci.*, **44**: 1259-1263.
- OTTAWAY, J. R., 1973. Some effects of temperature, desiccation, and light on the intertidal anemone *Actinia tenebrosa* Farquhar (Cnidaria: Anthozoa). *Aust. J. Mar. Freshwater Res.*, **24**: 103-126.
- PARKER, G. H., 1917. Pedal locomotion in actinians. *J. Exp. Zool.*, **22**: 111-124.
- PEARSE, V. B., 1974. Modification of sea anemone behavior by symbiotic zooxanthellae: Expansion and contraction. *Biol. Bull.*, **147**: 641-651.
- SIEGEL, R. W., 1960. Hereditary endosymbiosis in *Paramecium bursaria*. *Exp. Cell Res.*, **19**: 239-252.
- STANIER, R. Y., AND G. COHEN-BAZIRE, 1957. The role of light in the microbial world: some facts and speculations. *Symp. Soc. Gen. Microbiol.*, **7**: 56-89.
- TREMBLEY, A., 1744. *Mémoires pour servir à l'histoire d'un genre de polypes d'eau douce, à bras en forme de cornes*. Durand, Paris, 661 pp.
- TRENCH, R. K., 1971a. The physiology and biochemistry of zooxanthellae symbiotic with marine coelenterates. I. The assimilation of photosynthetic products of zooxanthellae by two marine coelenterates. *Proc. Roy. Soc. London Series B*, **177**: 225-235.
- TRENCH, R. K., 1971b. The physiology and biochemistry of zooxanthellae symbiotic with marine coelenterates. II. Liberation of fixed ^{14}C by zooxanthellae *in vitro*. *Proc. Roy. Soc. London Series B*, **177**: 237-250.
- TRENCH, R. K., 1971c. The physiology and biochemistry of zooxanthellae symbiotic with marine coelenterates. III. The effect of homogenates of host tissues on the excretion of photosynthetic products *in vitro* by zooxanthellae from two marine coelenterates. *Proc. Roy. Soc. London Series B*, **177**: 251-264.
- VANDERMEULEN, J. H., N. D. DAVIS, AND L. MUSCATINE, 1972. The effect of inhibitors of photosynthesis on zooxanthellae in corals and other marine invertebrates. *Mar. Biol.*, **16**: 185-191.
- WHITNEY, D. D., 1907. Artificial removal of the green bodies of *Hydra (Chlorohydra) viridis*. *Biol. Bull.*, **13**: 291-299.
- WILSON, E. B., 1891. The heliotropism of *Hydra*. *Amer. Natur.*, **25**: 413-433.
- ZAHL, P. A., AND J. J. A. McLAUGHLIN, 1959. Studies in marine biology. IV. On the role of algal cells in the tissues of marine invertebrates. *J. Protozool.*, **6**: 344-352.